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Comparison of belowground biomass in C_3- and C_4-dominated mixed communities in a Chesapeake Bay brackish marsh

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Abstract

Belowground biomass is a critical factor regulating ecosystem functions of coastal marshes, including soil organic matter (SOM) accumulation and the ability of these systems to keep pace with sea-level rise. Nevertheless, belowground biomass responses to environmental and vegetation changes have been given little emphasis marsh studies. Here we present a method using stable carbon isotopes and color to identify root and rhizomes of *Schoenoplectus americanus* (Pers.) Volk. ex Schinz and R. Keller (C_3) and *Spartina patens* (Ait.) Muhl. (C_4) occurring in C_3– and C_4-dominated communities in a Chesapeake Bay brackish marsh. The functional significance of the biomass classes we identified is underscored by differences in their chemistry, depth profiles, and variation in biomass and profiles relative to abiotic and biotic factors. C_3 rhizomes had the lowest concentrations of cellulose (29.19%) and lignin (14.43%) and the lowest C:N (46.97) and lignin:N (0.16) ratios. We distinguished two types of C_3 roots, and of these, the dark red C_3 roots had anomalously high C:N (195.35) and lignin:N (1.14) ratios, compared with other root and rhizome classes examined here and with previously published values. The C_4-dominated community had significantly greater belowground biomass (4119.1 g m^{-2}) than the C_3-dominated community (3256.9 g m^{-2}), due to greater total root biomass and a 3.6-fold higher C_3-root:rhizome ratio in the C_4-dominated community. C_3 rhizomes were distributed significantly shallower in the C_4-dominated community, while C_3 roots were significantly deeper. Variability in C_3 rhizome depth distributions was explained primarily by C_4 biomass, and C_3 roots were explained primarily by water table height. Our results suggest that belowground biomass in this system is sensitive to slight variations in water table height (across an 8 cm range), and that the reduced overlap between C_3 and C_4 root profiles in the C_4-dominated community may account for the greater total root biomass observed in that community. Given that future elevated atmospheric CO_2 and accelerated sea-level rise are likely to increase C_3 abundance in Atlantic and Gulf coast marshes, investigations that quantify how patterns of C_3 and C_4 belowground biomass respond to environmental and biological factors stand to improve our understanding of ecosystem-wide impacts of global changes on coastal wetlands.

Abbreviations: SOM – soil organic matter; C – carbon; CO_2 – carbon dioxide; N – nitrogen; $\delta^{13}C$ – ^{13}C abundance relative to standard Peedee Belemnite; SERC – Smithsonian Environmental Research Center

Introduction

Coastal wetlands such as salt and brackish marshes provide a range of ecosystem services, including shoreline protection through soil organic matter (SOM) accumulation, storing and filtering of toxic compounds from riverine inputs, exporting organic nutrients to estuarine ecosystems, and serving as habitat for fish and wildlife (Vernberg, 1993). Accelerated sea-level rise and

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increasing atmospheric CO_2 concentrations may increase inundation, erosion, and saltwater intrusion while also altering the production and distribution of dominant marsh plant species (Scavia et al., 2002). Studies of coastal salt and brackish marshes have documented environmental impacts related to sea-level rise, including submergence (e.g., Broome et al., 1995; Burdick et al., 1989; Parrondo et al., 1978) and salinity (e.g., Bradley and Morris, 1992; Seliskar, 1985; Webb and Mendelssohn, 1996); however, only a small fraction of such studies have addressed belowground biomass. Peat soils in Gulf and mid-Atlantic coastal marshes often consist mostly of root- and rhizome-derived SOM (Kearney et al., 1988; Mitsch and Gosselink, 1993), and production of belowground biomass has been hypothesized to regulate nutrient cycling, vertical accretion and the ability of marsh systems to keep pace with sea-level rise (Morris and Bowden, 1986; Pont et al., 2002; Rybczyk et al., 1998; Scheffer and Aerts, 2000). To develop models of vegetation change in coastal marshes and their effects on soil accumulation, it would be valuable to know how patterns of C_3 and C_4 belowground biomass vary as a function of abiotic factors and biological factors. In this paper, we present detailed belowground biomass data on dominant C_3 and C_4 species that co-occur in a Chesapeake Bay brackish marsh, and we investigate the extent to which environmental and biological factors alter biomass characteristics.

Marshes with mixtures of C_3 and C_4 plant species are a common feature in Atlantic and Gulf coast marshes and provide a unique opportunity to investigate detailed characteristics of and the factors influencing belowground biomass. C_3 and C_4 species have different stable isotopic signatures of carbon, providing a means to distinguish species-specific sources of SOM (e.g., Choi et al., 2001) and potentially species-specific belowground biomass. C_3 and C_4 species also respond differently to abiotic factors, including flooding and salinity (Broome et al., 1995; Naidoo et al., 1992; Ross and Chabreck, 1972) as well as rising atmospheric CO_2 (Bazzaz, 1990; Drake, 1992; Owensby et al., 1999) and increased temperatures (Ehleringer et al., 1991). A thorough understanding of belowground biomass must also account for deep roots. It has been suggested that roots of the C_3 brackish marsh sedge *Schoenoplectus americanus* may tap fresh water as deep as 100 cm (Arp et al., 1993). Most studies of root biomass, however, examine roots only in the top 30-cm of soil, and very few studies have adequately examined variability in belowground biomass in deeper soil layers. Variability in deep root biomass may have disproportionate impacts on ecosystem functions such as SOM accumulation and nutrient cycling, as waterlogging of soils alters decomposition rates of marsh litter by as much as two orders of magnitude as a function of soil depth (Benner et al., 1984; Pozo and Colino, 1992).

In this study, we quantify and develop explanatory models of both the inter- and intraspecific variation in belowground biomass of *S. americanus* (a C_3 sedge) and *Spartina patens* (a C_4 grass) in a C_3-dominated and a C_4-dominated mixed community in a Chesapeake Bay brackish marsh. A unique aspect of our study site is the abundance of long-term data (1987–Present) on *in situ* physiological- to ecosystem-level responses of these species to elevated atmospheric CO_2 (Drake, 1992; Drake et al., 1996), including CO_2 interactions with salinity (Rasse et al., 2005). Our study is motivated in part by the need to establish a protocol for analyzing belowground biomass at this experimental site. The objectives of this study are (1) to develop a methodological approach to identify belowground biomass of the C_3 and C_4 plants by species; (2) to quantify biomass chemistry, as the polymeric components of organic matter and the ratios C:N and lignin:N; (3) to compare total biomass of roots and rhizomes of each species in C_3-dominated and C_4-dominated mixed communities; (4) to quantify depth profiles of roots and rhizomes to 65 cm; and (5) to test hypotheses about the extent to which variation in biomass and depth profiles is explained by environmental variation vs. biomass of co-existing species.

Materials and methods

Description of the study site

This study was conducted in two plant communities in a brackish marsh bordering the Rhode River, a sub-estuary in the Chesapeake Bay (38°51′ N, 76°32′ W). The two dominant species

at the study site, *Schoenoplectus americanus* and *Spartina patens*, are common to marshes on the Atlantic and the Northern Gulf coasts and occur in both mixed and monoculture communities. Other species that occur in areas peripheral to our study plots include *Distichlis spicata* (L.) Greene, *Typha angustifolia* L., *Hibiscus moscheutos* L., *Scirpus robustus* Pursh, and *Iva frutescens* L. The marsh is 40–60 cm above mean low water, and 20% of high tides flood the marsh (Jordan et al., 1986). Tidal amplitude in the Rhode River is 30 cm, although events such as storms can create more extreme water levels. The soil salinity ranges from < 1 to 20 ppt, the lowest levels occurring in spring and the highest levels occurring in the fall. Weather records from a meteorological station ca. 1 km north of the marsh show mean annual rainfall is 120 cm and mean annual temperature is 12 °C.

The research was partly motivated by the need to establish a protocol for analyzing belowground biomass and soil excavated from an ongoing CO_2 enrichment experiment at our study site. Since 1987, researchers at the Smithsonian Environmental Research Center (SERC, Edgewater, MD) have employed 30 open top chambers set at ambient and elevated (700 ppm) CO_2 to measure physiological, community, and ecosystem variables in three community types: a C_3 community, a C_4 community, and a mix of mostly (~70%) C_4 plants with some C_3 plants (Leadley and Drake, 1993). Our study was started in 1997, two years prior to excavating and analyzing soils from the CO_2 study. Objectives for both studies include separating belowground biomass to species, quantifying chemical properties of belowground biomass, and quantifying biomass stocks and depth profiles of biomass.

We collected soil and plant samples from five 1.5-m^2 plots in a C_3-dominated community and six 1.5-m^2 plots in a C_4-dominated community. We included six plots in the C_4-dominated community in order to control for the greater variation in biomass in that community. The C_3-dominated community was located ~15 m southwest of the C_3 community in the CO_2 enrichment experiment, and the C_4-dominated community was located ~15 m northeast of the C_4 and mixed communities in the CO_2 enrichment experiment. In May 1997, we selected plots within these two communities with the intention of replicating the vegetation in the CO_2 experiment while at the same time representing contrasting levels of C_4- vs. C_3-dominance. Accurately replicating the plant composition in the "C_3 community" of the CO_2 experiment was not possible, however, given substantial changes in species composition over the 17-year duration of the experiment, with some plots becoming almost exclusively C_4 while others remaining C_3-dominated. As a matter of focus, in this study we selected only plots that were truly C_3-dominated for our C_3-dominated community. Plots were chosen based on the following criteria: (1) Percent of area covered by C_4 grasses; (2) Density of C_3 stems (# stems m^{-2}); (3) Maximum canopy height (cm); (4) Maximum litter height (cm); and (5) The number of shrubs (*Iva frutescens*) within a 2-m radius. A more detailed description of the vegetation characteristics at our study site can be found in Drake et al. (1986) and Saunders (2003).

Soil coring and sampling intervals

Duplicate piston-cores 70 cm in length and 5.1 cm in diameter were taken from each plot at three month intervals starting in March 1998 and ending in June 1999. These cores were considerably deeper than cores used in previous root biomass studies as prior work suggested *S. americanus* roots could tap fresh water as deep as 100 cm (Arp et al., 1993) and pilot cores taken in 1997 showed considerable root biomass at depths between 30 and 75 cm depth. The first set of cores taken in March 1998 yielded root biomass estimates with relatively low variability (CV generally $< 25\%$ for all root classes), but rhizome biomass with far higher variability (CV = 40–80% for both rhizome classes). For this reason, the additional cores taken after March 1998 were used primarily for separating rhizomes. The decision to focus our root-sorting effort on rhizome biomass made the power of our statistical tests for rhizome biomass comparable to root biomass.

Although the primary goal of this study was to understand how depth-dependent patterns of root biomass vary with abiotic and biotic variables rather than to capture time-dependent patterns (e.g., peak root biomass), there was only a 7% difference between our estimate of root biomass in March 1998 in the C_3-dominated community and at peak season (August, 1999) in

the ambient chambers of the CO_2 enrichment experiment. In addition, the CV of total root biomass in March 1998 (CV = 14–19%) was similar to that of the CO_2 enrichment experiment (CV = 15.9%). Nevertheless, to further examine the potential for time-dependent changes in the variability in root biomass at our plots, we also sampled roots (0–12.5 cm only) in a subset of plots (3 per community) in March 1999 and June 1999. These data were used to determine the degree of autocorrelation between seasons (i.e., March vs. June) and seasonal changes in the magnitude of biomass variability. Finally, to address potential time-dependent changes in the variation of biomass with depth, we compared the depths of maximum root biomass (hereafter referred to as "peak depths") for March 1998 with those for August 1999 in the CO_2 enrichment experiment.

Separating plant material from soil

Soil cores were cut into 2.5-cm segments centered at 2.5, 5, 7.5, 12.5, 15, 22.5, and 25 cm depth, and 5-cm segments were cut at depths 35, 45, 55 and 65 cm. Segment width was measured using digital calipers. Soil segments were washed over a 1-mm screen, and the remaining material was hand-separated into roots, rhizomes and a miscellaneous litter fraction. An attempt was made to identify living roots and rhizomes; therefore, roots and rhizomes that were mostly damaged (i.e., a frayed or broken epidermis or stele), smaller roots (1–2 cm in length) that were flaccid, and all root and rhizome material less than 1-cm in length were placed in the litter fraction. Root and rhizomes were oven-dried at 60 °C to a constant dry weight (approximately two weeks) and weighed. Biomass of each soil segment was divided by the segment width and converted to units of g dry weight cm^{-3}.

To categorize roots and rhizomes as either C_3 (*S. americanus*) or C_4 (*S. patens*), we explored two complementary methods. The first method involved visually separating roots and rhizomes into different color classes. *Schoenoplectus americanus* roots and rhizomes are typically orange, red, or dark red, with a small proportion (<7%, see Results) of white or yellow roots. *Spartina patens* biomass is mostly white or gray. We separated roots and rhizomes of distinctly different colors from a block of soil in the mixed community into five classes of "standards" labeled as "white roots," "red roots," "dark red roots," "white rhizomes" and "red rhizomes." The standards were placed in sealed bags filled with deionized water and refrigerated throughout the duration of the separation process. All roots and rhizomes separated from the soil cores were compared to the standards and categorized according to the color they most closely matched.

The second method to categorize belowground biomass involved using isotopic signatures of root and rhizome carbon. Carbon isotopic signatures, in per mil units (‰), were determined by the following equation:

$$\delta^{13}C_{sample} = [(R_{sample}/R_{standard}) - 1] \times 1000, \quad (1)$$

where R_{sample} is the $^{13}C/^{12}C$ ratio of the sample, and $R_{standard}$ is the $^{13}C/^{12}C$ ratio of a standard relative to Pee-Dee Belemnite. All $\delta^{13}C$ values were obtained by combusting 2–3 mg subsamples in an elemental analyzer (NA1500 Series 1, Carlo Erba Instrumentazione) and measuring $\delta^{13}C$ on a SIRA Series II isotope ratio mass spectrometer. $\delta^{13}C$ analyses were performed by Larry Giles at Duke University.

Root and rhizome isotopic signatures were used to calculate the percent of biomass belonging to the C_3 species (referred to hereafter as "$\%C_3$"). $\%C_3$ was calculated by the dual end-member equation:

$$\%C_3 = 100^{*}(\delta^{13}C_{sample} - \delta^{13}C_{C4}) / (\delta^{13}C_{C3} - \delta^{13}C_{C4}), \quad (2)$$

where $\delta^{13}C_{sample}$ is the isotopic signature of a given sample of biomass, $\delta^{13}C_{C3}$ is the isotopic signature of pure *S. americanus* biomass and $\delta^{13}C_{C4}$ is the isotopic signature of pure *S. patens* biomass. Pure C_3 biomass was collected in a C_3 monoculture within 10 m of the C_3-dominated plots, and pure C_4 biomass was collected in a C_4 monoculture 10 m east of the C_3 community in the CO_2 experiment. $\delta^{13}C$ of wetland plant species has been shown to vary as a function of salinity, soil moisture, and soil nutrients (Ewe and Sternberg, 2003; Lin and Sternberg, 1992). Although pure material was not collected in

replicate, we conducted a sensitivity analysis to determine the potential error in the estimated $\%C_3$ associated with intraspecific variation in $\delta^{13}C_{sample}$ of our study species. Estimated values of $\%C_3$ ranged from 0 to 100%. For all but the white roots, $\%C_3$ was estimated on biomass collected from two mixed plots and two pure C_3 plots ($n=4$). Because both C_3 and C_4 species produced white roots, we expected relatively greater variability in the $\%C_3$ of white roots compared to the other classes. We therefore determined the $\%C_3$ of white roots for five plots in the C_4-dominated community and three plots in the C_3-dominated community ($n=8$). Biomass for these analyses was obtained by pooling samples (over all depths) from the March 1998 cores. All samples were homogenized using a Wiley Mill prior to isotopic analysis.

Chemical analysis

Previous studies have shown that chemical components of plant litter are important factors controlling litter decomposition rates (Aber et al., 1985; Scheffer and Aerts, 2000; Valiela et al., 1985). In July 1999, we separated an additional 3–5 g of the five root and rhizome color classes from soil excavated ($15 \times 15 \times 40$ cm deep) from three of the plots in the C_4-dominated community. Freeze-dried samples were homogenized using a Wiley Mill, and the chemical components of the samples were determined for each root and rhizome class using a sequential extraction method (Wieder and Star, 1998). These analyses included four extractions to quantify the proportion of total organic matter as nonpolar solubles, hot water solubles, holocellulose, and lignin. The extraction method also produced a residual component, since for all samples, the sum of the proportions totaled less than 1. Percent C and N of root and rhizome classes were measured using an elemental analyzer (NA1500 Series 1, Carlo Erba Instrumentazione). C:N ratios were expressed as mass ratios (i.e., %C of dry sample / %N of dry sample) and not molar ratios in order to facilitate comparison of our results with previously published mass ratios of C:N (e.g., Ball and Drake, 1997; Curtis et al., 1989, 1990; Gordon and Jackson, 2000). The lignin:N ratio was calculated by dividing the % lignin of organic matter by the %N of dry sample.

Estimating root and rhizome biomass

Root and rhizome biomass (expressed as g dry weight m^{-2}) was estimated for each plot by interpolating and then integrating biomass profiles from 0 to 65 cm depth. In addition to calculating the total biomass of each color class, we determined the total biomass of C_3-derived white roots and C_4-derived white roots separately (hereafter referred to as "C_3-white roots" and "C_4-white roots", respectively). For each plot, C_3-white root biomass was determined by multiplying total white root biomass by the $\%C_3$ of white root biomass, the latter obtained from the isotopic signature of white roots described in equation 2. The $\delta^{13}C$ of white root biomass was not determined for two of the C_3-dominated plots and one of the C_4-dominated plots, so the community-averaged $\%C_3$ value was used for those plots instead.

Depth profiles

Biomass of each root and rhizome class was plotted vs. depth to compare community differences in biomass density (g cm^{-3}) at each depth and the overall shape of the profile. To aid in comparing community differences in the shapes of the profiles, we used two approaches. First, profiles of each root and rhizome class were fitted to a gamma density function. The gamma density function can reproduce a variety of profile shapes, including an exponential distribution, an asymmetric unimodal distribution, a normal distribution, or a uniform distribution. The S-plus function *nlminb* was used to obtain maximum likelihood estimates of the two parameters in the gamma density function for each profile. For each community, the function was fitted to profiles of mean biomass and used strictly as a qualitative aid to visualize community differences in profile shape and the peak depth of each biomass type.

In the second approach, we quantified the 1st, 2nd, and 3rd quartiles of the biomass as a function of depth. The 1st quartile depth is defined as the depth (in cm) above which 25% of the total biomass occurs. Likewise, the 2nd and 3rd quartile depths contain 50% and 75% of total

biomass, respectively. Quartile depths were computed for each root and rhizome class in each individual plot and used as response variables for statistical analysis.

Soil oxygen and water table measurements

Measurements of the soil oxygen depth and water table height were used as correlates with biomass and depth profile metrics. Water table height (cm above the marsh surface; <0 for cm below the soil surface) was measured several times in each plot (approximately 25 observations per plot; $n = 276$ total) between June 1997 and December 1999. These data were used to develop plot-specific regressions predicting water table height as a function of water level in the adjacent Rhode River estuary. Water levels have been monitored continuously at the SERC dock since 1986. Water level data were then used as inputs for the regression models in order to reconstruct daily water table heights from January 1998 to June 1999. For each plot water table height was averaged over January to March 1998 and over January 1998 to June 1999 to be used as a covariate for root and rhizome data, respectively.

The depth of rust formation on steel rods (hereafter referred to as "soil oxygen depth") was used to measure the depth (in mm; <0 for mm below the soil surface) at which reducing conditions predominated in the soil (Bridgham et al., 1991). In each plot, two replicate steel rods were inserted in the sediment and left in place for at least three weeks. These measurements were made in August 1998 and again in April 1999. Measurements from August 1998 were used as covariates for root data, and the average values of the August 1998 and April 1999 data were used as covariates for the rhizome data.

Statistical analyses

Chemical components, C:N ratios, and lignin:N ratios were analyzed with an analysis-of-variance (ANOVA) and *post-hoc* Least Squares (LS) means comparisons to determine differences between the five root and rhizome classes. Level of significance for testing was Bonferroni-corrected for the number of classes ($\alpha = 0.05/5$, $P < 0.01$).

Between-community differences in biomass and root:rhizome ratios were analyzed using an ANOVA and *post-hoc* comparisons of LS means. We used a Repeated-Measures ANOVA (RMANOVA) and *post-hoc* LS means comparisons to determine community differences in biomass at each soil depth. For all of these analyses, the probability of Type I error was set at 0.05 (i.e., $\alpha = 0.05$).

Simple linear regressions were used to examine the relationships between biomass and three explanatory variables (other species root + rhizome biomass, water table height, and soil oxygen depth) and between the three quartile depths and four explanatory variables (other species root biomass, other species rhizome biomass, water table height and soil oxygen depth). Initial analyses using aboveground biomass as a covariate showed no significant relationships and therefore were omitted from our results. Quadratic terms were included where significant to determine whether the relationship changed with an increase in the predictor variable. Stepwise linear regression was then used to determine which of the explanatory variables controlled biomass and quartile depths. Variables that were non-significant ($P > 0.15$) were excluded from the model. Variation in quartile depths of white roots was expected to primarily reflect differences in $\%C_3$; therefore, quartile depths were regressed only against the $\delta^{13}C$ of white root biomass and no other predictor variables.

ANOVA, RMANOVA, *post-hoc* LS means comparisons, and regressions were all performed using SAS 9.0 (SAS Institute Inc., Cary, NC).

Results

Species identification and chemistry of belowground biomass

Developing a method to identify belowground biomass by species is a necessary prerequisite to quantifying the intra- and interspecific variability in biomass characteristics *in situ*. Color classes and isotopic ($\delta^{13}C$) signatures were utilized here to identify roots and rhizomes of the dominant C_3 (*Schoenoplectus americanus*) and C_4 (*Spartina patens*) species that co-occur in a Chesapeake Bay brackish marsh. For the most part, the $\delta^{13}C$

values of the different color classes confirm that these classifications correspond to either the C_3 or C_4 species (Table 1). Red and dark red roots and red rhizomes are of C_3 origin, and the white rhizomes are of C_4 origin. Only the white roots are ambiguous with respect to species origin. Although primarily of C_4 origin, the white roots may include a substantial percentage of C_3 roots, 22.7% in the C_3-dominated community and 9.7% in the C_4-dominated community (Table 1). Overall, the C_3-white roots account for 7% and 3% of the total C_3 root biomass in the C_3- and C_4-dominated communities, respectively.

Because of the potential for intraspecific variation in carbon isotopes, we determined the sensitivity of the dual end-member equation (2) in calculating species composition. As a test case, we focused on C_3 red rhizomes. We found that the mean $\delta^{13}C$ of red rhizome samples was 1.1 per mil lower than that of pure material (Table 1), and we attributed this difference to intraspecific variation in $\delta^{13}C$ of red rhizomes given the easily identifiable morphology of the red rhizomes. The 1.1 per mil difference between the sample and pure material therefore provides an indication of the potential variability in $\delta^{13}C$ of C_3 biomass. In equation 2, this difference in $\delta^{13}C$ changes the estimated $\%C_3$ by 9%.

Several inter- and intra-specific differences were observed in root and rhizome chemistry (Table 2). Compared to the C_4 (*S. patens*) rhizomes, C_3 (*S. americanus*) rhizomes had more hot water solubles (29.27% vs. 8.72%), less cellulose (29.19% vs. 55.45%) and lignin (14.43% vs. 26.95%), and lower C:N (46.97 vs. 109.62) and lignin:N ratios (0.16 vs. 0.63). We also found intraspecific differences in the chemistry of C_3 biomass. C_3 red and dark red roots both had more holocellulose and lignin and had higher C:N and lignin:N ratios than the C_3 rhizomes. Red and dark red roots also differed significantly with respect to their C:N and lignin:N ratios, the dark red roots having the highest C:N and lignin:N ratios (195.35 and 1.14, respectively) compared to all other root and rhizome classes (46.97–116.64 and 0.16–0.71, respectively).

Table 1. Stable isotopic signatures ($\delta^{13}C$) and calculated species composition ($\%C_3$) of the five color classes of roots and rhizomes (means ± standard error)

Color Class	$\delta^{13}C$ of biomass	$\delta^{13}C$ of pure material	Calculated $\%C_3$
White roots (C_4-dominated)	−13.30 (0.24)	−12.18	9.72 (2.09)
White roots (C_3-dominated)	−14.80 (0.43)	−12.18	22.71 (3.75)
Red roots	−23.54 (0.26)	−23.69	98.01 (1.99)
Dark red roots	−24.54 (0.06)	−24.37	100.00 (0.00)
White rhizomes	−13.68 (0.16)	−12.82	7.38 (1.35)
Red rhizomes	−25.53 (0.38)	−24.44	100.00 (0.00)

$\%C_3$ is calculated using a dual end-member equation (Equation 2, Materials and Methods). "$\delta^{13}C$ of biomass" represents biomass separated from March 1998 soil cores, and "$\delta^{13}C$ of pure material" represents C_3 and C_4 plant biomass collected nearby *in situ*. For all classes, $n = 4$, except white roots. White roots were analyzed separately for the C_4-dominated community ($n = 5$) and the C_3-dominated community ($n = 3$).

Table 2. Percent of total organic components, lignin:N ratio and C:N ratio of roots and rhizomes (means ± standard error, $n=3$). Biomass is identified as C_3 or C_4 (in parentheses) according to its $\delta^{13}C$ signature

	White roots (mostly C_4)	Red roots (C_3)	Dark red roots (C_3)	White rhizomes (C_4)	Red rhizomes (C_3)
Oils, Waxes	9.06 (0.40) ba	10.81 (0.16) a	10.11 (0.48) a	7.40 (0.50) b	7.66 (0.57) b
Hot water solubles	5.27 (0.70) b	6.43 (0.34) b	5.28 (0.24) b	8.72 (1.44) b	29.27 (0.77) a
Holocellulose	51.68 (2.01) ab	47.81 (1.64) b	48.45 (0.88) ab	55.45 (0.81) a	29.19 (1.45) c
Lignin	30.41 (0.51) a	26.50 (0.64) b	26.27 (0.44) b	26.95 (1.28) ab	14.43 (0.38) c
Residual	3.58 (1.72) bc	8.45 (1.29) b	9.88 (0.46) b	1.48 (0.93) c	19.45 (1.92) a
Lignin:N	0.54 (0.02) b	0.71 (0.05) b	1.14 (0.09) a	0.63 (0.06) b	0.16 (0.03) c
C:N	82.56 (3.44) bc	116.64 (7.58) b	195.35 (16.89) a	109.62 (14.64) b	46.97 (10.77) c

Different letters indicate significant differences between root and rhizome classes.

Table 3. Biomass (g dry weight m^{-2}) of root and rhizome classes (means ± standard error[a]) in the C_3-dominated and C_4-dominated communities. In each community, the biomass of white roots was calculated separately for the C_3 and C_4 species, using the mean $\%C_3$ (see Table 1) of white roots in the given community. $P < 0.05$ indicates a significant difference between communities

	C_3-Dominated community	C_4-Dominated community	*P*
Roots (March 1998)			
C_3-white roots	87.2 (15.8)	53.4 (10.9)	ns
C_4-white roots	286.2 (24.1)	507.6 (55.3)	<0.05
Total white roots	373.4 (38.8)	561.0 (56.5)	<0.05
Red roots (C_3)	578.2 (52.3)	693.4 (65.8)	ns
Dark red roots (C_3)	544.4 (34.3)	784.8 (114.0)	ns
Total roots	1496.0 (92.0)	2039.2 (154.2)	<0.05
Rhizomes (March 1998–June 1999)			
White rhizomes (C_4)	143.3 (38.6)	1435.8 (102.2)	<0.001
Red rhizomes (C_3)	1643.7 (150.4)	576.2 (136.1)	<0.001
Total rhizomes	1787.0 (172.8)	2011.9 (65.9)	ns
Total roots + Rhizomes	3256.9 (222.9)	4119.1 (202.3)	<0.05

[a]Rhizome biomass values for a given plot were averaged over all sampling dates (from March 1998 to June 1999) in order to calculate a value for each plot separately; therefore, the standard error presented here represents the error associated with the community mean rather than the error associated with different sampling dates.

Biomass of roots and rhizomes

Total root and rhizome biomass was significantly greater in the C_4-dominated community than the C_3-dominated community (Table 3). This difference was explained by a significantly greater (+36%) root biomass in the C_4-dominated community and a non-significant trend of greater rhizome biomass in the C_4-dominated community. Although C_3 rhizome biomass was higher in the C_3-dominated community than the C_4-dominated community, C_3 root biomass was not significantly higher. Consequently, the C_3-root:rhizome ratio was 3.6 times higher ($F_{1,9} = 16.07$, $P < 0.005$) in the C_4-dominated community.

Analyses of root biomass in the top 12.5-cm of soils sampled in March 1999 and June 1999 indicated that depth-dependent variability in March root biomass, for the most part, fairly represented the variability in growing season biomass. Autocorrelation in root biomass between sample dates was high for the white roots ($R^2 = 0.90$, $P < 0.004$) and red roots ($R^2 = 0.96$, $P < 0.0006$), consistent with the fact that the biomass rankings (in the top 12.5-cm) were the same from plot to plot for both seasons. Autocorrelation between seasons for the dark red roots, however, was not significant ($R^2 = 0.02$, ns). The magnitude of variability, measured as the coefficient of variation (CV), was similar for root biomass between March 1999 and June 1999. The CV of root biomass was 5.9% and 8.5% for C_3- and C_4-dominated communities, respectively, in March 1999, and was 5.8% and 5.6%, respectively, in June 1999.

Depth profiles

For both C_3 and C_4 species, we found consistent differences between their root and rhizome profiles. Rhizomes of both species were restricted to the upper 15 cm of soil, whereas roots were detected as deep as 65 cm (Figure 1). With the exception of C_3 rhizomes in the C_4-dominated community, rhizome biomass of both species was greatest at depths of 5 and 7.5 cm, whereas the greatest root biomass was found either at 2.5, 5, or 15 cm, depending on the class of root and community type.

Several between-community differences were also observed within each root and rhizome class. White root biomass in the upper 5 cm of soil of the C_4-dominated community was three- to five-fold greater than in the C_3-dominated community. Both types of C_3 roots appeared to have more biomass allocated to deeper soil layers in the C_4-dominated community, with significantly greater red and dark red root biomass in the C_4-dominated community at 22.5 and 25 cm, respectively. Between-community differences in

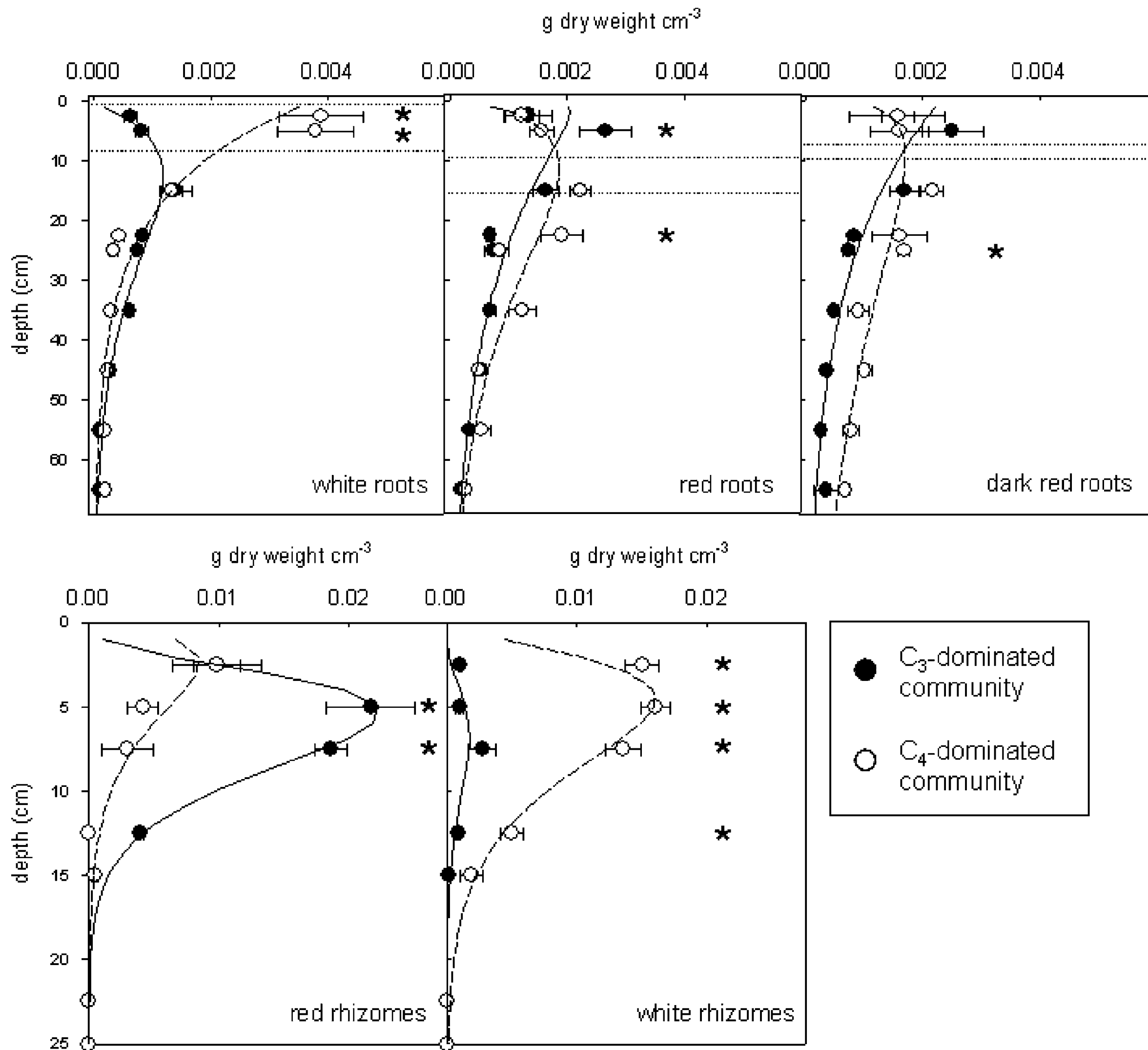

Figure 1. Depth profiles of root and rhizome classes in C_3-dominated (black circles) and C_4-dominated (white circles) communities. Asterisks indicate significant differences ($P < 0.05$) between communities. Mean biomass values for each root and rhizome class were fitted to a gamma density function to clarify profile differences between communities (C_3-dominated community = solid lines; C_4-dominated community = dashed lines). Horizontal dotted lines indicate 95% confidence intervals for the peak depths of root biomass from samples taken in August, 1999 in the C_3 community of the CO_2 enrichment study.

rhizome profiles were also observed, with both C_3 and C_4 rhizomes allocated deeper in the C_3-dominated community than in the C_4-dominated community.

Root profiles measured in the "C_3 community" of the CO_2 experiment (sampled in August, 1999) provided a means to assess whether root profiles changed between seasons. The peak depth of white roots in August 1999 ranged from 0.58 to 8.42 cm, while peak depths of red and dark red roots were substantially deeper, ranging from 9.6 to 15.4 cm and 7.3 to 9.7 cm, respectively (Figure 1). Although these peak roots depths differed from those in the C_3-dominated community in the March 1998 sampling, they closely matched peak root depths in the C_4-dominated community, where white roots had shallower profiles compared to the red and dark red roots (Figure 1). This agreement was not altogether unexpected, as white C_4 rhizome biomass

was substantial in the C_3 community of the CO_2 experiment, accounting for 42% of total rhizome biomass. In comparison, white rhizome biomass accounted for 71% of total rhizome biomass in our C_4-dominated community (compared to 7% in the C_3-dominated community, Table 2).

Effects of biotic and abiotic factors on biomass and depth profiles

The observed variability in root biomass was explained primarily by abiotic factors, whereas rhizome biomass was explained by a combination of both abiotic and biotic factors (Table 4, Figure 2). C_4-white root biomass was negatively correlated with water table height, but red root biomass had a significant nonlinear relationship with water table height (Figure 2). For both white and red root classes, water table height (including both the main effect plus second-order term in the case of red roots) explained over 80% of the variation in biomass (Table 4). None of the factors explained dark red root biomass. C_3 rhizome biomass was significantly and inversely correlated with total C_4 biomass (Figure 2, Table 4). C_4 rhizomes were signficantly and negatively correlated with both C_3 biomass ($R^2 = 0.81$, $P < 0.0005$) and water table height ($R^2 = 0.84$, $P < 0.0001$), when the two variables were considered separately (Figure 2). However, the partial determination coefficients for these variables were relatively small ($R^2 < 0.08$,

Table 4. Root and rhizome biomass of C_3 and C_4 species as a function of other species biomass (roots + rhizomes), water table depth, and soil oxygen depth, as determined by stepwise multiple regression. Variables with the superscript "2" indicate a squared term (+ main effect) was included in the final regression model

Variable	R^2	F	df_{model}, df_{error}	P
C_4-White roots[a]				
C_3 biomass	ns[b]			
Water table	0.85	65.57	1,5	< 0.0005
O_2 depth	0.18	14.25	1,5	< 0.05
Overall F test	0.94	36.23	2,5	< 0.005
Red roots (C_3)				
C_4 biomass	ns			
Water table	na[c]			
Water table^2	0.79	36.61	1,8	< 0.0005
O_2 depth	ns			
Overall F test	0.83	19.14	2,8	< 0.001
Dark red roots (C_3)				
C_4 biomass	ns			
Water table	ns			
O_2 depth	ns			
Overall F test	ns			
Red rhizomes (C_3)				
C_4 biomass	0.83	44.02	1,9	< 0.0001
water table	ns			
O_2 depth	ns			
Overall F test	0.83	44.02	1,9	< 0.0001
White rhizomes (C_4)				
C_3 biomass	0.05	3.34	1,8	0.1049
Water table	0.08	5.67	1,8	< 0.05
O_2 depth	ns			
Overall F test	0.89	31.95	2,8	< 0.0005

[a]C_4-white root biomass calculated using $\delta^{13}C$ of white root biomass (n = 8). For all other root and rhizome classes, n = 11.
[b]ns = Variable not significant ($P > 0.15$).
[c]na = When higher order terms are significant, partial R^2 and F tests are omitted for the main effect.

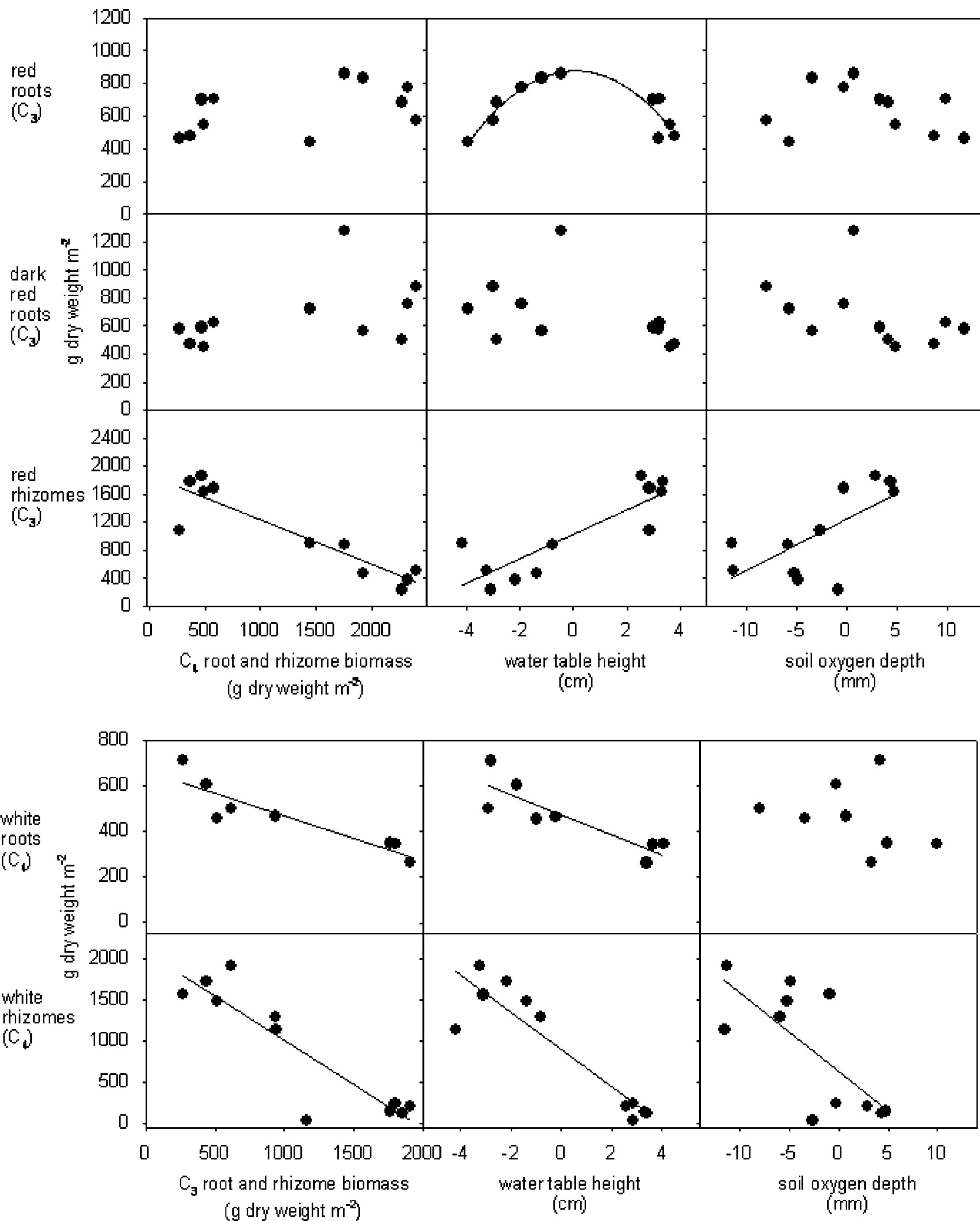

Figure 2. Root and rhizome biomass vs. biomass of the competing species, water table height, and soil oxygen depth. Statistically significant ($P < 0.05$) simple linear regression lines are also shown for each relationship. Presence of a quadratic line indicates a statistically significant change in the relationship of biomass with that factor.

Table 4); therefore, it was not possible to determine which of these was the primary variable explaining C_4 rhizome biomass.

Depth profiles of the white roots correlated strongly with $\delta^{13}C$, a measure of species composition (Table 5, Figure 3). More negative $\delta^{13}C$ values were associated with deeper quartile depths (Figure 3), confirming the deeper rooting behavior of C_3-white roots compared to C_4-white roots. Because of this strong influence of species composition on depth profiles of white roots, additional covariates were not tested.

Depth profiles of the other root and rhizome classes were largely explained by the same factors that explained the variation in biomass. Water table height explained 59% and 50% of the variation in the 1st quartile depths of the C_3 red and dark red roots, respectively (Table 5). For both root classes, increased water table height was associated with shallower rooting profiles (Figure 4). For the C_3 rhizomes, the 1st and 2nd quartiles were explained by both water table height and C_4 biomass while the 3rd quartile depth was primarily explained by C_4 root biomass (Table 5). Linear regression plots showed that depth distributions of the C_3 rhizomes were shallower with increased C_4 root biomass and lower water table height (Figure 4). Stepwise regression showed that 1st and 2nd quartiles of C_4 rhizome biomass significantly

Table 5. Quartiles of root and rhizome profiles as a function of other species biomass (as roots or rhizomes), water table depth, and soil oxygen depth, as determined by stepwise multiple regression. Quartiles of white roots are shown as a function of $\delta^{13}C$ only

Variable	1st Quart.				2nd Quart.				3rd Quart.			
	R^2	F	df_{num}, df_{den}	P	R^2	F	df_{num}, df_{den}	P	R	F	df_{num}, df_{den}	P
White roots												
$\delta^{13}C$	0.84	32.52	1,6	0.0013	0.84	31.11	1,6	0.0014	0.81	25.56	1,6	0.0023
Overall *F* test	0.84	32.52	1,6	0.0013	0.84	31.11	1,6	0.0014	0.81	25.56	1,6	0.0023
Red roots												
C_4 roots	ns				ns				ns			
C_4 rhizomes	ns				ns				ns			
Water table	0.59	12.71	1,9	0.0061	0.27	3.27	1,9	0.1042	ns			
O_2 depth	ns				ns				ns			
Overall *F* test	0.59	12.71	1,9	0.0061	0.27	3.27	1,9	0.1042	ns			
Dark red roots												
C_4 roots	0.08	3.11	1,7	0.1212	ns				ns			
C_4 rhizomes	ns				ns				ns			
Water table	0.50	19.63	1,7	0.0030	0.65	16.98	1,9	0.0026	0.71	21.58	1,9	0.0012
O_2 depth	0.15	5.78	1,7	0.0472	ns				ns			
Overall *F* test	0.82	10.80	3,7	0.0051	0.65	16.98	1,9	0.0026	0.71	21.58	1,9	0.0012
Red rhizomes												
C_4 roots	0.17	3.41	1,7	0.1073	ns				0.58	15.40	1,8	0.0044
C_4 rhizomes	0.19	3.11	1,7	0.1214	ns				ns			
Water table	0.30	5.47	1,7	0.0520	0.45	7.22	1,9	0.0249	ns			
O_2 depth	ns				ns				0.32	8.39	1,8	0.0200
Overall *F* test	0.61	3.68	3,7	0.0708	0.45	7.22	1,9	0.0249	0.70	9.21	2,8	0.0084
White rhizomes												
C_3 roots	ns				ns				ns			
C_3 rhizomes	0.30	3.94	1,9	0.0785	0.24	2.91	1,9	0.1223	ns			
Water table	ns				ns				ns			
O_2 depth	ns				ns				ns			
Overall *F* test	0.30	3.94	1,9	0.0785	0.24	2.91	1,9	0.1223	ns			

ns = Variable not significant ($P > 0.15$).

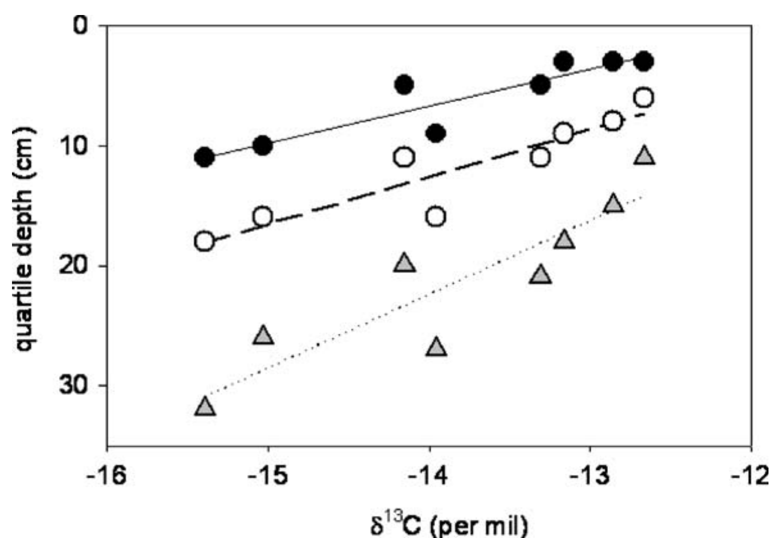

Figure 3. Depths of 25, 50, and 75% quartiles of white roots as a function of the carbon isotopic signature ($\delta^{13}C$) of white root biomass. 25% quartiles are represented by black circles, 50% quartiles by white circles, and 75% quartiles by gray triangles. Simple linear regression lines are also shown (solid lines, 25% quartiles; long-dashed lines, 50% quartiles; and dotted lines, 75% quartiles).

increased in depth with increasing C_3 rhizome biomass (Table 5) although the determination coefficient was ≤30% in each case.

Discussion

Species identification and chemistry of belowground biomass

Identifying roots and rhizomes by species is key to understanding how different species respond to biotic and abiotic factors in a natural system. We have demonstrated two complementary methods that use color classes and isotopic ($\delta^{13}C$) signatures to identify roots and rhizomes of the dominant C_3 (*Schoenoplectus americanus*) and C_4 (*Spartina patens*) species that co-occur in a Chesapeake Bay brackish marsh. With the exception of white roots, the color classes that we used largely agree with the known isotopic ($\delta^{13}C$) signatures of the roots and rhizomes of the two species. One advantage of separating roots and rhizomes by color is that it adds little additional time to the process of separating biomass from soil and requires no substantial costs for subsequent analyses, such as with isotopic analysis. In addition, separating roots into several color classes may further elucidate intraspecific as well interspecific variation in ecosystem functional characteristics. For example, we have found substantial differences in the chemistry and depth profiles of red and dark red C_3 roots (discussed below). We recommend, however, that isotopic analysis be used as a supplementary tool (e.g., on a subset of biomass samples), to assess the validity of color classes as a proxy for species at a given study site and to clarify biomass classes that cannot be identified to species by color alone (e.g., white roots).

One important caveat to the use of isotopes in identifying root and rhizome biomass to species is that isotopic signatures may vary within a species as a function of environmental conditions (Ewe and Sternberg, 2003; Lin and Sternberg, 1992). Because our two study species use different photosynthetic (C_3 vs. C_4) pathways and therefore have contrasting carbon isotopic signatures, the dual end-member mixing model used to identify biomass to species worked relatively well: all but one root and rhizome class was quantified as ≥90% C_3 or C_4 in origin. Nevertheless, we found that intraspecific variation in the $\delta^{13}C$ of C_3 rhizomes was likely to vary as much as 1.1 per mil, sufficient to alter estimated $\%C_3$ by 9%. To limit the effects of intraspecific variation, we recommend that future studies should account for potential spatial variability between

sites (e.g., due to variation in salinity, moisture, and nutrients) and temporal variability between seasons and years.

The functional significance of the types of root and rhizome biomass identified here is underscored by differences in their chemical composition. Of all root and rhizome classes analyzed, the C_3 rhizomes had the highest proportion of hot water solubles, the lowest proportion of lignin, the lowest C:N ratio, and the lowest lignin:N ratio (Table 3). Each of these characteristics has been shown to increase decomposition of marsh plant litter (Ball and Drake, 1997; Scheffer and Aerts, 2000; Valiela et al., 1985). The high proportion of hot water solubles also suggests that the C_3 rhizomes serve

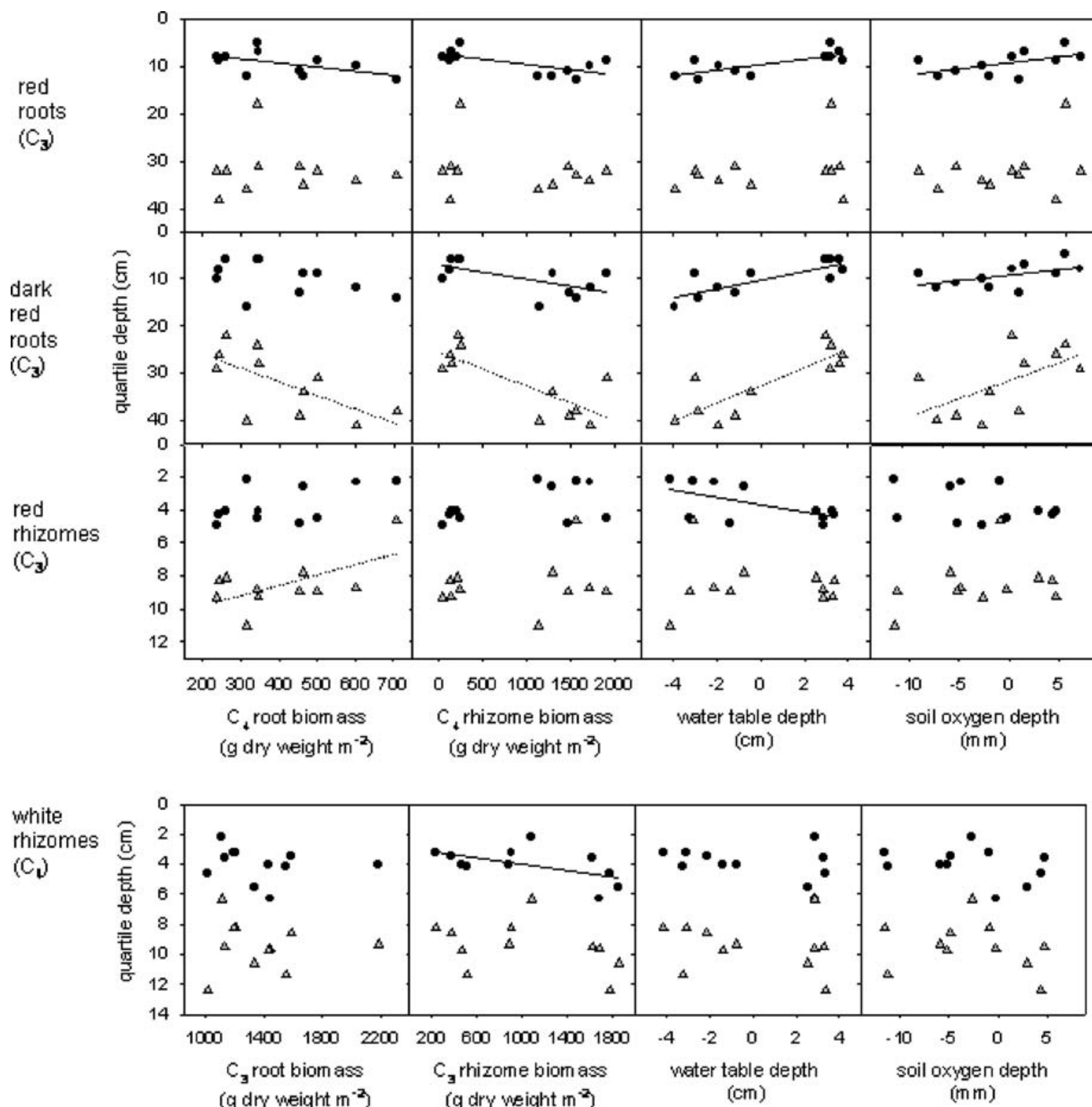

Figure 4. Depths of 25 and 75% quartiles of roots and rhizomes as a function of other species root biomass, other species rhizome biomass, water table depth, and soil oxygen depth. 25% quartiles are represented by black circles, 75% quartiles by gray triangles. Simple linear regression lines are also shown (solid lines, 25% quartiles; dotted lines, 75% quartiles) when significant ($P < 0.05$).

as storage for carbohydrates. Given their relatively low C:N ratio, these rhizomes may also serve as storage for nitrogen, a trait common to sedge rhizomes (Scheffer and Aerts, 2000).

In contrast to the C_3 rhizomes, the dark red roots of the C_3 species had the highest lignin:N and C:N ratios, suggesting that these roots may have the slowest decomposition rates and an overall important regulatory role in plant nutrient cycling. The darker color of these roots and their relatively larger cross-sectional diameters (C. Saunders, pers. obs.) are traits consistent with being older and possibly more senescent (Comas et al., 2000; Krauss and Deacon, 1994). The C:N ratio of the dark red roots (195.4) is anomalously high compared to belowground biomass in Atlantic coast brackish and salt marshes (26–48, Gallagher and Plumley, 1979; 50, White and Howes, 1994) and terrestrial systems in general (43–79, Gordon and Jackson, 2000). A high C:N ratio may indicate that nitrogen translocation occurs as C_3 roots senesce. Curtis et al. (1989) found that senescing *S. americanus* shoots have relatively high nitrogen recovery efficiencies (50–75%) compared to *S. patens* (30%). C_3 rhizomes therefore probably provide a sink for nitrogen recovered from both above- and belowground tissues.

Variation in root and rhizome biomass and depth profiles

Our findings present some similarities but also some contrasts with previous studies examining biomass responses of these species to biotic and abiotic factors (e.g., Broome et al., 1995; Schwarzbeck, 1982; Seliskar, 1990). We found C_4 root and rhizome biomass correlated negatively with increasing water levels, consistent with experimental findings by Broome et al. (1995), and with field observations showing lower *S. patens* biomass and greater *S. americanus* biomass in the lower elevations at our site (Arp et al., 1993). Broome et al. (1995) found that water table did not affect *S. americanus* aboveground biomass. In contrast, water table height was the primary factor explaining the observed variation in both C_3 and C_4 root biomass at our site. We observed a significant nonlinear relationship between C_3 red root biomass and water table height, although none of the factors explained dark red C_3 root biomass. C_3 rhizome biomass was not significantly correlated to water table height but rather inversely correlated with C_4 biomass. In summary, our findings suggest that both biotic and abiotic factors may have different effects on production and allocation to aboveground biomass, rhizomes, and roots. Specifically, *S. americanus* roots may be more responsive to water depth as a result of their presence in deeper, anaerobic soil.

We also found that the depth profiles of *S. americanus* belowground biomass correlated significantly with water table and *S. patens* belowground biomass. *Spartina patens* has been described as a superior competitor because it forms a dense mat of roots and rhizomes in the top 5 cm of soil, potentially excluding other species (Bertness and Ellison, 1987; Gallagher and Plumley 1979; Schwarzbeck, 1982). Schwarzbeck (1982) observed that rhizomes of both *Spartina alterniflora* Loisel. (C_4) and *Juncus roemerianus* Scheele (C_3) had shallower depth profiles in the presence of *S. patens*. Similarly, we found that *S. americanus* rhizomes also had shallower depth profiles as a function of increased *S. patens* (root) biomass. Schwarzbeck (1982) showed that in the presence of *S. patens, S. alterniflora* rhizomes were more likely to grow toward the surface and form more shoots than in monocultures. Schwarzbeck (1982) argued that the relatively low physical strength of *S. alterniflora* shoots prevented it from penetrating the mat of *S. patens* from below. Given the tendency for *Schoenoplectus* spp. shoots to break under wave or wind stress (Coops and Vandervelde, 1996; Coops et al., 1994; C. Saunders, pers. obs.), the same mechanism could apply for *S. americanus*.

Although *S. americanus* rhizomes were shallower in the presence of *S. patens*, *S. americanus* roots were actually deeper in the presence of *S. patens*. The observed variability in root depth profiles was explained primarily by water table height (Table 5). These results confirm earlier experimental work showing that *S. americanus* roots have shallower depth profiles under waterlogged vs. drained conditions (Seliskar, 1990). The discrepancy between root and rhizome depth profile patterns, however, seems consistent with our analysis of the factors explaining *S. americanus* root and rhizome biomass: that *S. americanus* rhizomes are

more influenced by *S. patens* biomass, and *S. americanus* roots are more influenced by water table variation and consequent changes in the soil environment.

Poor aerenchyma development in *S. patens* roots limits this C_4 species in tolerating waterlogged conditions (Burdick, 1989; Burdick and Mendelssohn, 1990). Although we were unable to analyze the depth profile of *S. patens* roots, the depth profiles of *S. patens* rhizomes showed the weakest correlation with the explanatory variables that we tested (r^2 = 0.30, Table 5; Figure 4), possibly reflecting the inability of this species to supply sufficient oxygen to deeper roots and rhizomes in the areas dominated by presence of the C_3 species. This finding is consistent with observations by Schwarzbeck (1982) who found no difference in the depth distribution of *S. patens* rhizomes between pure *S. patens* communities and mixed communities of *S. patens* with *S. alterniflora* or *J. roemerianus*.

The variation in biomass, chemistry, and depth profiles of C_3 and C_4 belowground biomass may have important implications for our understanding of ecosystem responses to global changes. However, it is important to recognize the assumptions and limitations of the data presented. First, although the C_3 and C_4 rhizome biomass presented here is representative of biomass stocks averaged over an entire year, root biomass was only measured (over the top 60-cm) in March, rather than at the peak of the growing season (June–August). Ideally, we would like to obtain root biomass several times per year, but given the time consuming nature of separating roots from peat soils and the detailed depth resolution required for this study (at the expense of time resolution), this was not possible, nor necessary to address the goals of the study. Nevertheless, our analysis of a smaller subset of root data (top 12.5 cm depth, from six of the 11 plots) obtained in March and June of the following year showed significant autocorrelation (R^2 > 0.90) for C_4 white roots and C_3 red roots; in other words, the ranking of root biomass was the same from plot to plot between seasons. Because the variation in these root types is consistent between seasons, the effects of abiotic and biotic factors on these roots types discussed above (and summarized in Table 4) are likely to be applicable beyond the March sampling date. On the other hand, dark red (C_3) root biomass was not autocorrelated, but then the biomass of these roots also was not significantly explained by any of the predictor variables (Table 4).

A second limitation of our study is that our analysis of root profiles also applies to profiles measured in March. A comparison of peak depths of roots measured in August 1999 in the C_3 community of the adjacent CO_2 experiment (in the ambient CO_2 treatment) actually appears more consistent with peak depths roots that we measured in our C_4-dominated community. In our study, we have found that profiles of all three root classes were highly dependent on community composition. Although it has been repeatedly referred to as a "C_3 community" (e.g., Drake, 1992; Rasse et al., 2005), the C_3 community of the CO_2 experiment contains almost equal amounts of C_3 and C_4 rhizome biomass; thus, a comparison of the CO_2 experimental data with the C_4-dominated community may be more appropriate.

With the above limitations in mind, our study of the variability in biomass and profiles of C_3 and C_4 roots and rhizomes could be useful for understanding aspects of ecosystem functioning in this marsh system. First, it is notable that water table height accounted for over half of the explained variance in C_3 root profiles, even though the range in water table height was less than 8 cm at our site. Between-community differences in C_3 root depth profiles in particular may be due to the relatively extensive aerenchyma in *S. americanus* roots, allowing them to penetrate deeper, waterlogged soil in the presence of other species (Arp, 1991; Seliskar, 1988). Thus, our findings suggest that future changes in water table height, due to a sea-level rise of 9–88 cm over the next century (Scavia et al., 2002), may considerably alter depth profiles in this system both as a result of inter- and intraspecific changes in root profiles. Second, the fact that C_3 roots were deeper in the soil in the presence of C_4 biomass may provide a mechanism to explain why total C_3 root biomass remained the same in both C_3- and C_4-dominated communities, and in turn why total belowground biomass was greatest in the C_4-dominated community. We suspect that deeper C_3 root profiles in the C_4-dominated community essentially reduce the degree of niche-overlap

and competition with C_4 roots, causing greater community-level use of resources such as soil nutrients or freshwater (Arp et al., 1993). This mechanism is important as it suggests that belowground production, and possibly SOM accumulation, may be highest in C_4-dominated communities.

In summary, we have provided a novel method to identify roots and rhizomes of two co-occurring C_3 and C_4 species in a brackish coastal marsh. The use of isotopes and color is not only useful in distinguishing biomass types by species, but also in elucidating important inter- and intra-specific differences related to organic chemistry of biomass and nutrient concentrations. Furthermore, biomass stocks and depth profiles of the different root and rhizome types were often explained by different abiotic and biotic factors. Previous studies have shown a negative effect of increased water level on C_4 aboveground biomass and no effect on C_3 aboveground biomass. We also found C_4 belowground biomass to be negatively affected by water level. However, biomass and depth profiles of C_3 roots were mostly explained by water level, in contrast to C_3 rhizome biomass and depth profiles, which were explained mostly by other species' biomass. Given that the range in water table height at our site is relatively small (8-cm) but still accounted for much of the variation in biomass and depth profiles of these species, our results suggest that sea-level rise is likely to alter patterns of belowground biomass in this system.

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